The salinity affected soil was investigated for the study of phosphate solubilization by bacteria and fungi. In the present study 87 soil sample were collected from salinity affected area of Amravati district (Daryapur, Bhatkuli, and Anjangaon), among these samples, 34 samples showed the ability to solubilize the inorganic insoluble phosphate. From the study it was observed that the fungi (Aspergillus spp., Penicillium spp.) have more solubilizing ability of inorganic insoluble phosphate than bacteria, i.e., B. cereus, B. megaterium, Bacillus subtilis, pseudomonas spp., Enterobacter spp., Hence the application of biofertilizer prepared by above mentioned fungi should be helpful to increase the crop yield by solubilizing large concentration of inorganic insoluble phosphate.

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**Keywords:** Phosphate Solubilizing Bacteria (PSB), Pikovaskayas agar, Biofertilizer

**INTRODUCTION**

Microorganisms have the ability to solubilize the insoluble phosphates and maintain the nutrient status of soil (Richardson, 2001). Microorganisms are central to the soil P cycle and play a significant role in mediating the transfer of P between different inorganic and organic soil P fractions, subsequently releasing available P for plant acquisition (McLaughlin et al., 1988 and Oberson et al., 2001). Phosphorus (P) is one of the major essential macronutrients for plant and is applied to soil in the form of phosphatic fertilizers. In soil inorganic and organic forms of phosphorus is present. The inorganic forms of the element in soil are compound of calcium, iron, aluminum and fluorine. The organic forms are compounds of phytins, phospholipids and nucleic acid which come mainly by way of decaying vegetation. Therefore, soils containing high organic matter are also rich in organic forms of phosphorus (Subbarao, 1982).

Amravati district of Maharashtra state in India is a part of alluvial valley of Purna basin. The district lies between latitude 20º, 37’ and 21º, 26’ N and longitudes 76º, 37’ and 78º, 27’ E in northeastern part of Maharashtra. The district covers an area of about 12,212 sq/km, out of which 3053 sq. km (25%) is covered by Purna.
alluvium. The salinity-affected area is 1756 sq. km. (58% of alluvium) and occurs along central part of river basin, which is located in northwestern part of the district. The salinity in shallow as well as deep groundwater on one hand and scarcity of surface storage due to low rainfall on the other makes the situation worse for management of water resources for both drinking irrigation and industrial use.

A large portion of soluble inorganic phosphate applied to the soil as chemical fertilizer is immobilized rapidly and becomes unavailable to plant (Gorstein, 1986). Microorganisms are involved in a range of processes that affect the transformation of soil phosphorous and are thus an integral part of the soil phosphorous cycle. In particular, soil microorganisms are effect in releasing phosphorous form inorganic and organic pools of total soil phosphorous through solubilization and mineralization (Hilda and Fraga, 1999).

The average soil phosphorus concentration is about 0.05 ppm and varies widely among soil. The phosphorus concentration required by most plants varies from 0.003 to 0.3 ppm and depends on the crop species and level of production maximum corn grain yield may be obtained with 0.01 ppm phosphorus, if the yield potential is low, but 0.05 ppm phosphorus is needed under high yield potential. Organic phosphorus represents about 50% of total phosphorus in soil and typically varies between 15 and 80% in most soils. Soil organic phosphorus decrease with depth and the distribution with depth also vary among soils. The phosphorus content of soil ranges from about 1-3%. Therefore, the quantity increases with increasing organic C and N. However, the C: P, N: P ratios are more variable among soils than the C: N ratio, (Miller and Donahue, 1990).

Most of the Indian soil (98%) is poor in availability phosphorus to plants (Gaur, 1987). Agricultural soils generally contain adequate amount of total phosphorus but the amount of plant available phosphorous is small (Singh and Kapoor, 1994). Therefore application of phosphatic fertilizers is essential for optimum crop yield. But the utilization efficiency of phosphate fertilizers by plant is only 20-25% largely due to its chemical fixation in soil (Dave et al., 2003).

It is generally accepted that the mechanism of mineral phosphate solubilization by Phosphate Solubilizing Bacteria (PSB) strains is associated with the release of low molecular weight organic acids (Goldstein, 1995; Kim et al., 1997) like formic acid, acetic acid, lactic acid, sulphuric acid and propionic acid, which through their hydroxyl and carboxyl group chelate the cations bound to phosphate, thereby converting it into soluble forms (Kpomblekou and Tabatabai, 1994). However, P-solubilization is a complex phenomenon, which depends on many factors such as nutritional, physiological and growth conditions of the culture (Reyes et al., 1999). There is experimental evidence to support the role of organic acids in minerals phosphate solubilization (Haldar et al., 1990).

The present investigation is carried out to study the performance of phosphate solubilizing microorganism in salinity affected area if Amravati district (Daryapur, Bhatkuli, and Anjangaon) Though lot of work have been made on phosphate solubilizing microorganism from different parts of India and Maharashtra but not much work has been done on phosphate solubilizing microorganism or on report is available on study of phosphate solubilizing microorganism in salinity affected area of Amravati district (Figure 1).
The objective of this study was to isolate and characterized PSB from the salinity affected area in Daryapur, Bhatkuli, and Anjangaon in Amravati district. As chemical fertilizers and phosphate solubilizing biofertilizer are used by farmer to increase the production in present days, there is necessity to have a comparative study on potential of native phosphate solubilizing microorganism, which will help to diagnose the need of phosphate solubilizing biofertilizer application in the Amravati district (Daryapur, Bhatkuli, and Anjangaon).

**MATERIALS AND METHODS**

**Sample Collection**
Total 87 soil samples were collected from salinity affected area of Amravati district (Daryapur, Bhatkuli, and Anjangaon) in sterilized container. The soil suspension was prepared by mixing 1 g of soil sample in 9 mL distilled water then supernatant was discarded and soil sample was point inoculated on previously prepared and sterilized pikovaskaya’s agar plates. Then the pikovaskaya’s agar plates were incubated at 28±2 °C for 24-48 h. And after completion of incubation time, Zone of phosphate solubilization was recorded. The colonies showing clear zone of solubilization were further subculture on pikovaskaya’s agar plates.

**Microscopic Study of Bacteria**
Size, shape, arrangement and gram’s nature of the isolates were studied for gram’s staining. Smear was prepared from the isolated culture on clean glass slide, heat fixed and stained. The stained smear was observed under microscope (Oil immersion lance-100x). The fungal isolates were identified up to generic level based on their colony morphology and microscopic examination as outlined in the manual of (Gilman, 1957)

**Identification of Bacterial Isolated Through Biochemical Test**
The PSBs isolated from salt affected soils were identified up to generic level based on
morphological and biochemical tests as specified in Bergey’s Manual of Determinative Bacteriology (Holt et al., 1994). Biochemical test were performed as suggested by (Garrity et al., 2001) which included following tests Grams, IMViC reaction, catalase test, starch hydrolysis test oxidation fermentation test, Phenyl alanine deamination test, Nitrate reduction, Gelatin hydrolysis test, Urea hydrolysis test, Dehydrogenase test, Casein hydrolysis test, Citrate utilization test, Indol production test, Triple Sugar Iron (TSI) test, Carbohydrate fermentation test (Glucose, Fructose, Sucrose, Arabinose, Mannitol , Lactose, Treholase, Galactose, Raffinose), Motility test, Endospore staining and capsule staining.

Phosphate Solubilization by Plate Assay

Solubilization of tricalcium phosphate was detected in Pikovskaya’s Agar medium (Sundara-Rao and Sinha., 1963) each isolate was point inoculated in at the center of Pikovskaya’s Agar plate and inoculated for 24 – 48 h the development of clear around the colony indicated phosphate solubilizing activity.

Observe the zone of solubilization and maser diameter around the colony

RESULTS

On the basis of cultural character, Morphological character and biochemical character phosphate solubilizing bacteria was identifying. Following character was compare with ‘BERGEY’S MANUAL’ and all phosphate solubilizing bacteria and fungi were identified. That is Bacillus cereus, Bacillus megaterium, Bacillus subtilis, pseudomonas spp., Micrococcus spp., Enterobacter spp., fungi (Aspergillus spp., Penicillium spp.)

Out of this isolate fungi (Aspergillus spp., Penicillium spp.) having efficiency of Phosphate solubilization was more as compare to other isolated phosphate solubilizing bacteria that is (285). But Enterobacter spp. having efficiency of Phosphate solubilization was less as compare to other isolated phosphate solubilizing bacteria that is (127). Efficiency of Phosphate solubilization was determined by plate assay using Pikovaskaya’s Agar Medium (Figure 2).

To Isolate Phosphate Solubilizing Bacteria and Fungi from Amravati District (Daryapur, Bhatkuli, and Anjangaon)

- Colonies showing zone of clearance were observed on Pikovskaya’s agar plates.
- The ability to solubilize precipitated phosphate was positively exhibited by Pseudomonas spp. Bacillus cereus, Bacillus megaterium, Bacillus subtilis, Micrococcus spp., Enterobacter spp., fungi (Aspergillus spp., Penicillium spp.).
- All phosphate solubilizing bacteria and fungi was selected and subculture on Pikovskaya’s agar plates for further studies.


% of Efficiency of PSB was calculated by using following formula

\[ \text{Efficiency of Phosphate Solubilization} = \frac{\text{Solubilization Diameter}}{\text{Diameter of Colony}} \times 100 \]

Table 1 represents the percentage efficiency of different PSB using Pseudomonas spp.
### Table 1: Efficiency of Phosphate Solubilization

<table>
<thead>
<tr>
<th>S. No</th>
<th>PSB Strain</th>
<th>Colony Diameter</th>
<th>Solubilization Diameter</th>
<th>% Efficiency 48 Hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pseudomonas spp.</td>
<td>0.9</td>
<td>1.6</td>
<td>177</td>
</tr>
<tr>
<td>2</td>
<td>Bacillus cereus</td>
<td>1</td>
<td>1.6</td>
<td>160</td>
</tr>
<tr>
<td>3</td>
<td>Pseudomonas spp.</td>
<td>0.8</td>
<td>1.4</td>
<td>175</td>
</tr>
<tr>
<td>4</td>
<td>Bacillus megaterium</td>
<td>1.1</td>
<td>1.8</td>
<td>163</td>
</tr>
<tr>
<td>5</td>
<td>Aspergillus spp</td>
<td>0.7</td>
<td>2</td>
<td>285</td>
</tr>
<tr>
<td>6</td>
<td>Penicillium spp.</td>
<td>0.8</td>
<td>1.6</td>
<td>200</td>
</tr>
<tr>
<td>7</td>
<td>Enterobacter spp.</td>
<td>1.1</td>
<td>1.4</td>
<td>127</td>
</tr>
<tr>
<td>8</td>
<td>Bacillus subtilis</td>
<td>0.9</td>
<td>1.4</td>
<td>155</td>
</tr>
<tr>
<td>9</td>
<td>Micrococcus spp.</td>
<td>0.7</td>
<td>1.4</td>
<td>200</td>
</tr>
<tr>
<td>10</td>
<td>Fungi (N. I.)</td>
<td>0.8</td>
<td>1.8</td>
<td>225</td>
</tr>
<tr>
<td>11</td>
<td>Fungi (N. I.)</td>
<td>1.1</td>
<td>2</td>
<td>180</td>
</tr>
</tbody>
</table>

### Figure 2: Result of Efficiency of Phosphate Solubilizer
**DISCUSSION**

Phosphate solubilizing bacteria survive well in the soils which provide organic substrates, nutrient, minerals, adequate moisture and tolerable environmental conditions. Efficient phosphate solubilizer always prefers soils which have good carbon for their survival.

From the salinity affected area of Amravati district. Total 87 soil sample from three different tahsil (Daryapur, Bhatkuli, and Anjangaon) were collected and the different species of phosphate solubilizing bacteria were isolated using pikovaskaya’s media. Out of 87 soil samples, 34 containing the different species of Phosphate solubilizing bacteria and fungi. Among these isolated Phosphate solubilizing bacteria, only Phosphate solubilizing bacteria were identified on the basis of following tests Grams, IMViC reaction, catalase test, starch hydrolysis test, oxidase test, Phenyl alanine deamination test, Nitrate reduction, Gelatin hydrolysis test, Urea hydrolysis test, Dehydrogenase test, Casein hydrolysis test, Citrate utilization test, Indol production test, Triple sugar iron (TSI) test, Carbohydrate fermentation test (Glucose, Fructose, Sucrose, Arabinose, Mannitol, Lactose, Treholase, Galactose, Raffinose), Motility test, Endospore staining and capsule staining.

Out of isolates 34 are bacteria and fungi in which 23 are of genus Bacillus, 4 are of *pseudomonas* spp. and remaining 7 are of fungi which are fungi (*Aspergillus* spp., *Penicillium* spp.) and other are not identified. Different species of bacillus like *Ba.cereus* are 11, *Ba.megaterium* are 7 and *Ba.subtilis* are 5 in number.

Among the bacteria the genus bacillus was dominating than *pseudomonas* spp., among the *Ba.cereus* is found to be the most dominating followed by *Ba.megaterium* and *Ba.subtilis*. In Daryapur tashil *Ba.subtilis* and fungi *Aspergillus* spp. were most dominating than *Ba.Megaterium* and *Penicillium* spp. whereas *pseudomonas* spp. completely absent. In Anjangaon tehsil *Penicillium* spp. and *Ba.Megaterium* is dominating followed by *pseudomonas* spp. *Ba.subtilis* and *Ba.cereus*. In Bhatkuli tehsil *pseudomonas* spp. is dominating followed by *Ba.Megaterium*, *Penicillium* spp, *Aspergillus* spp., *Ba.subtilis* and *Ba.cereus*.

Bilolkar *et al.* (1996) reported that *Bacillus* spp. was mostly dominating in the soil of Latur, Osmanabad and Parbhani whereas *pseudomonas* spp. was dominating in the soil from Aurangabad to Nanded district.

Bhattacharya *et al.* (1997) reported that bacteria were found as the predominant phosphate solubilizing microorganism in all Vidarba soil followed by fungi.

Production of enzyme like phosphates is other mechanism of phosphate solubilization (Rodriguez and Fraga, 1999). *Aspergillus* spp isolates, showed high activity of AP at 11 M of P; however, the production of this enzyme was under detection limit in excess of phosphate compared to limiting condition, which could explained that the synthesis of alkaline phosphatase by these bacteria was inducible in low Pi, while it was repressed in high concentration. These results are in concordance with solubilization activity Pikovaskaya’s Agar, where *Aspergillus* spp. isolates were strong P solubilizer. Interestingly, *Enterobacter* spp. strain produced a smaller drop
in pH value compared to others isolates. This might suggest that this strain is capable to solubilize phosphate by other ways than the production of organic acid. Therefore, we found a positive correlation between phosphate-solubilizing capacity and phosphatase enzyme activity.

CONCLUSION

It is concluded from the present study that all isolated phosphate solubilizing bacteria and fungi from salinity affected soil in Amravati district (Daryapur, Bhatkuli, and Anjangaon) are very useful for increasing solubilization of inorganic insoluble phosphate. This isolate are very important for increasing crop yield which is taken in salinity affected soil in productivity of, cotton, jawar, bazri, soybean (Glycine Max) etc. From the study it was observed that the fungi (Aspergillus spp, Penicillium spp.) have the more solubilizing ability of inorganic insoluble phosphate than bacteria, i.e., B.cereus, B.megaterium, pseudomonas spp, Enterobacter spp., Hence the application of biofertilizer prepared by above mentioned fungi should be helpful to increase the crop yield in salinity affected soil by solubilizing large concentration of inorganic insoluble phosphate.

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